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# Characterization of water-forming NADH oxidases for co-factor regeneration

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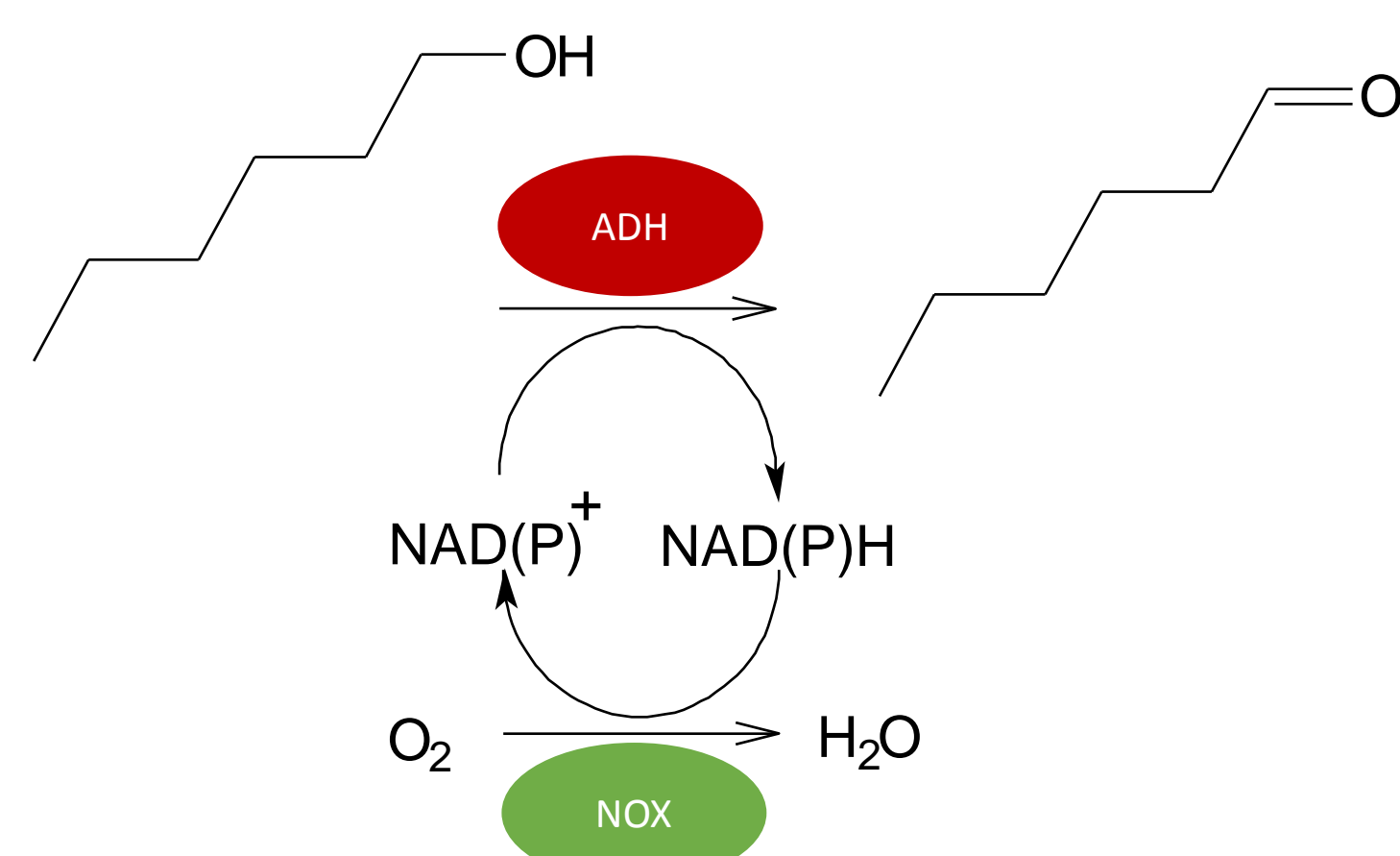
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Traditional chemical methods for alcohol oxidation are often associated with issues such as high consumption of expensive oxidizing agents, generation of metal waste and the use of environmentally undesirable organic solvents. Developing green, selective catalysts is therefore important from an environmental and economic perspective [1].

Alcohol dehydrogenases (ADH) offer one such alternative. However, the reaction requires the oxidized nicotinamide co-factor (NAD<sup>+</sup>) that must be recycled due to its high cost contribution. One regeneration method that offers certain advantages is the oxidation of NADH using water forming NADH oxidases (NOX-2). The implementation of the ADH/NOX system for alcohol oxidation, however, requires consideration of several different issues. Enzyme activity and stability at relevant pH and temperature conditions, but also the tolerance to the substrates and products present (alcohols and aldehydes) are important properties to characterize. Importantly, inactivation by gas-liquid interfaces has been reported for some enzymes, such as the NOX from *Lactococcus lactis* [2]. Thus, investigating the sensitivity to bubbling is also highly important from a process development perspective.

The oxidation of hexanol is used as a model reaction system:

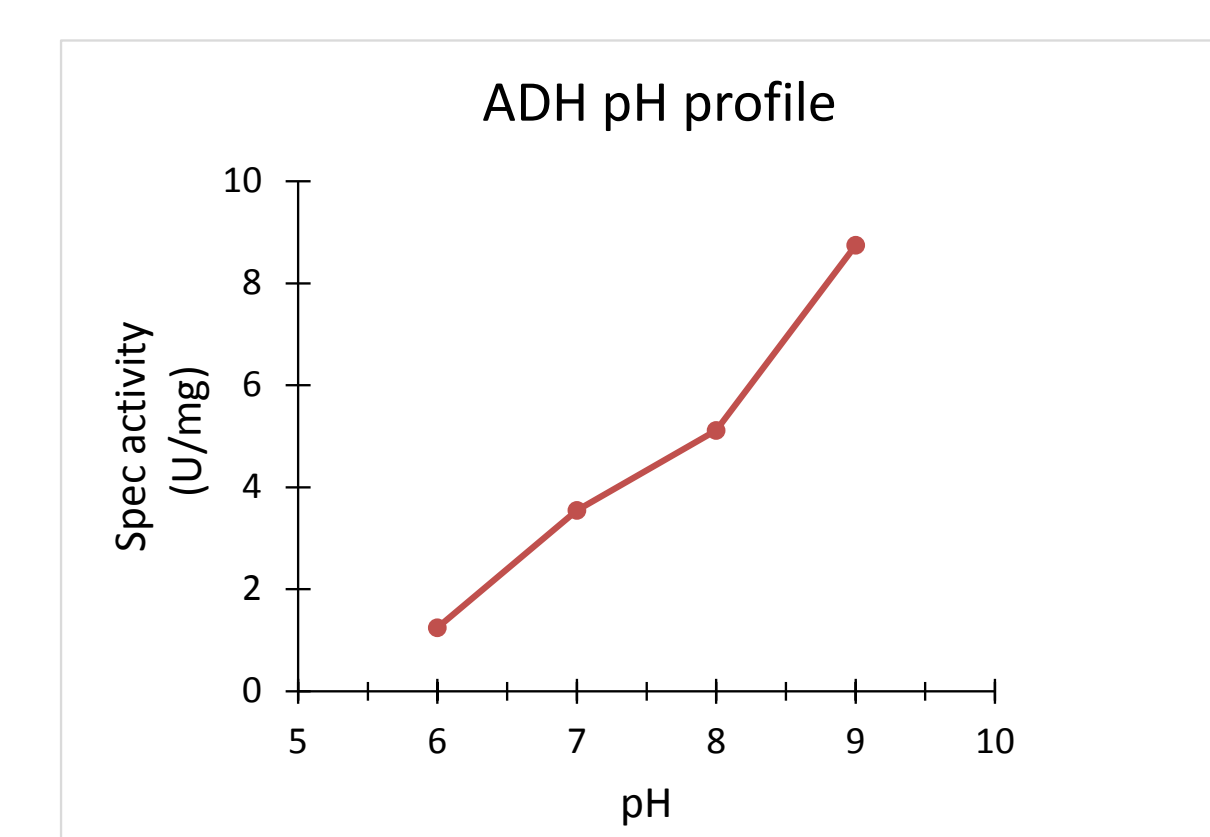
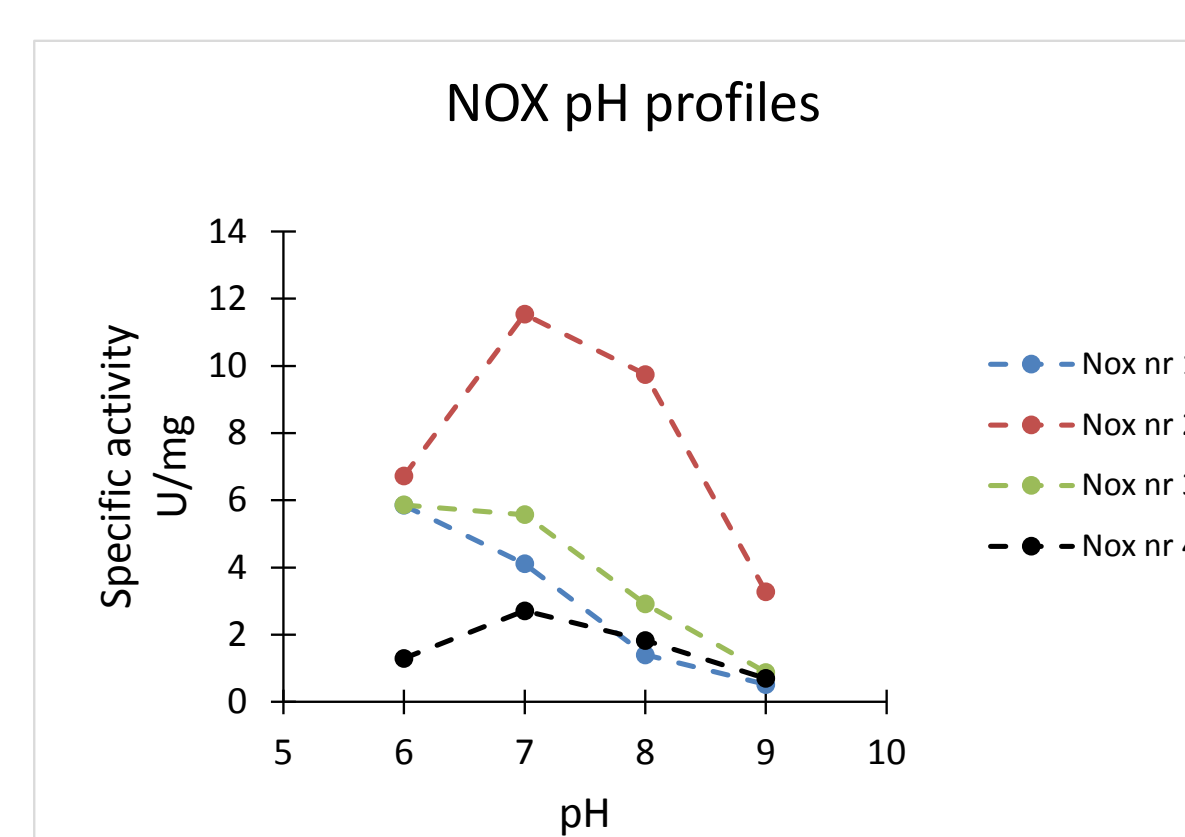


## NOX sensitivity to the gas-liquid interface

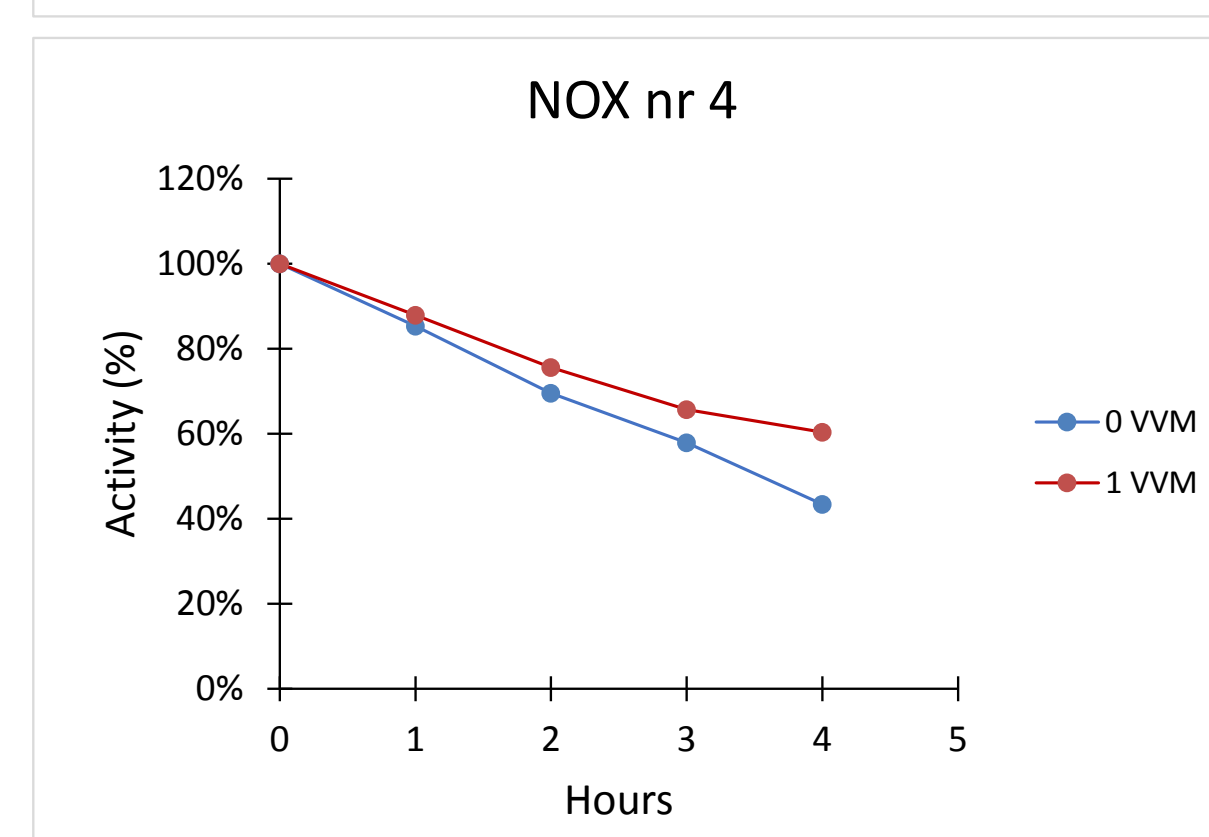
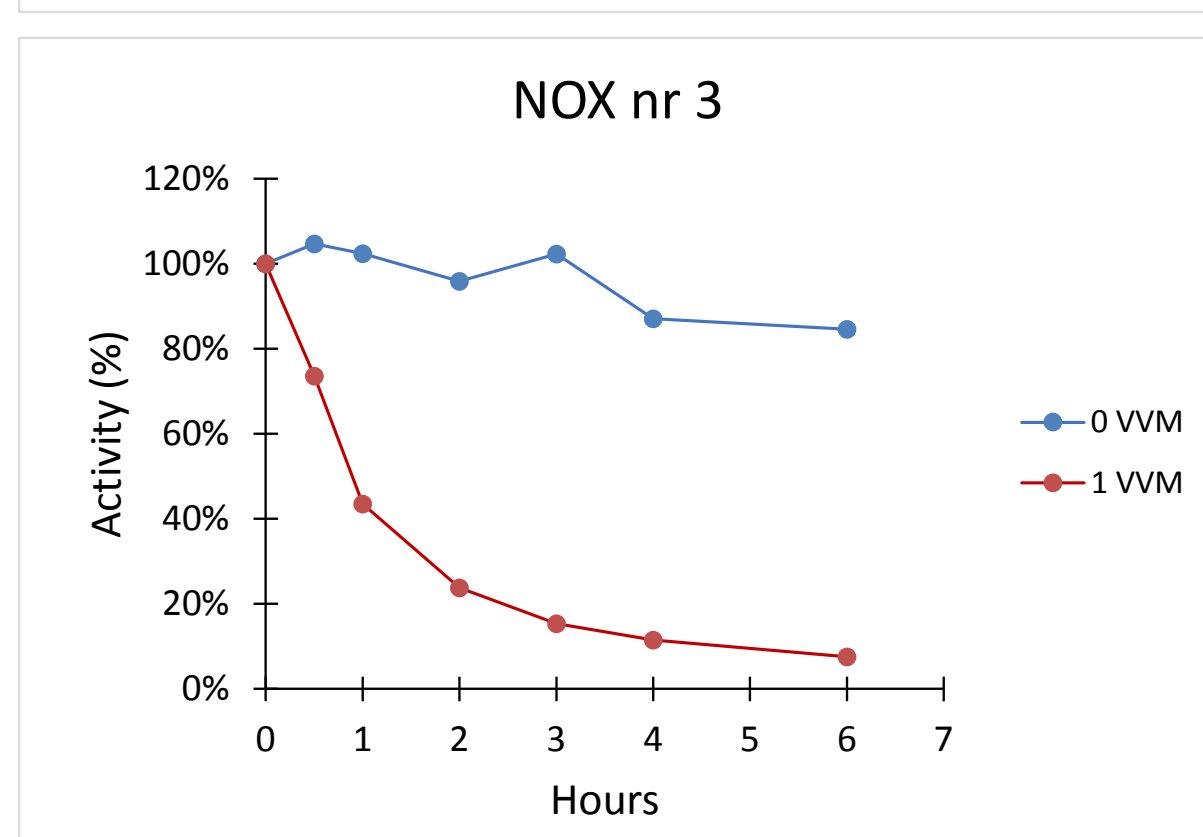
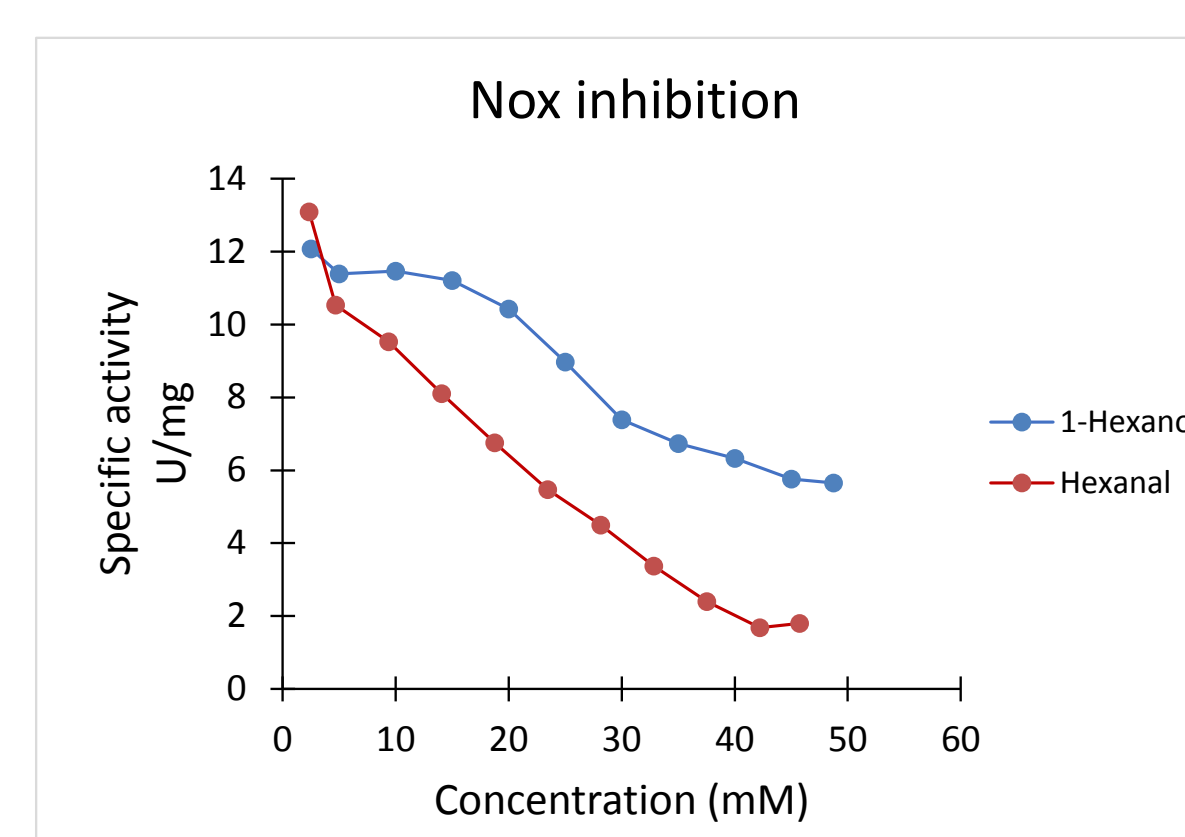
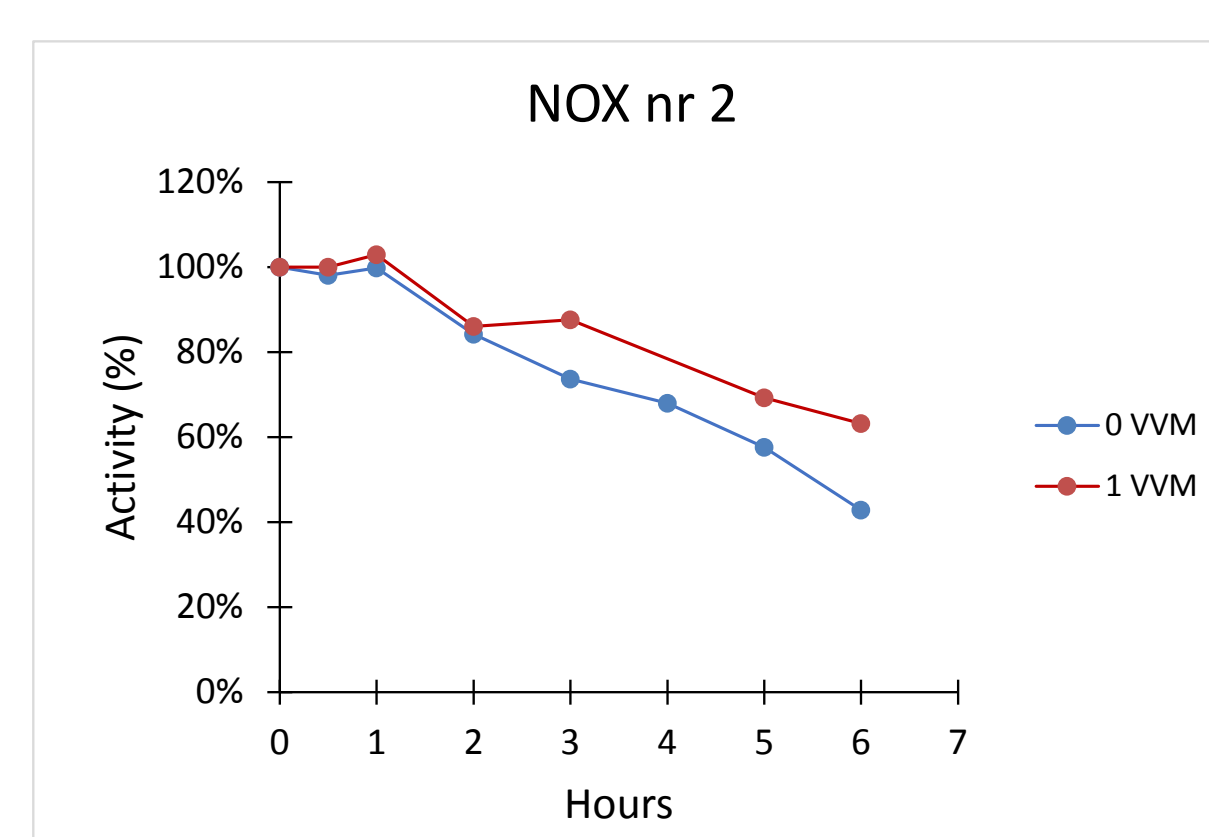
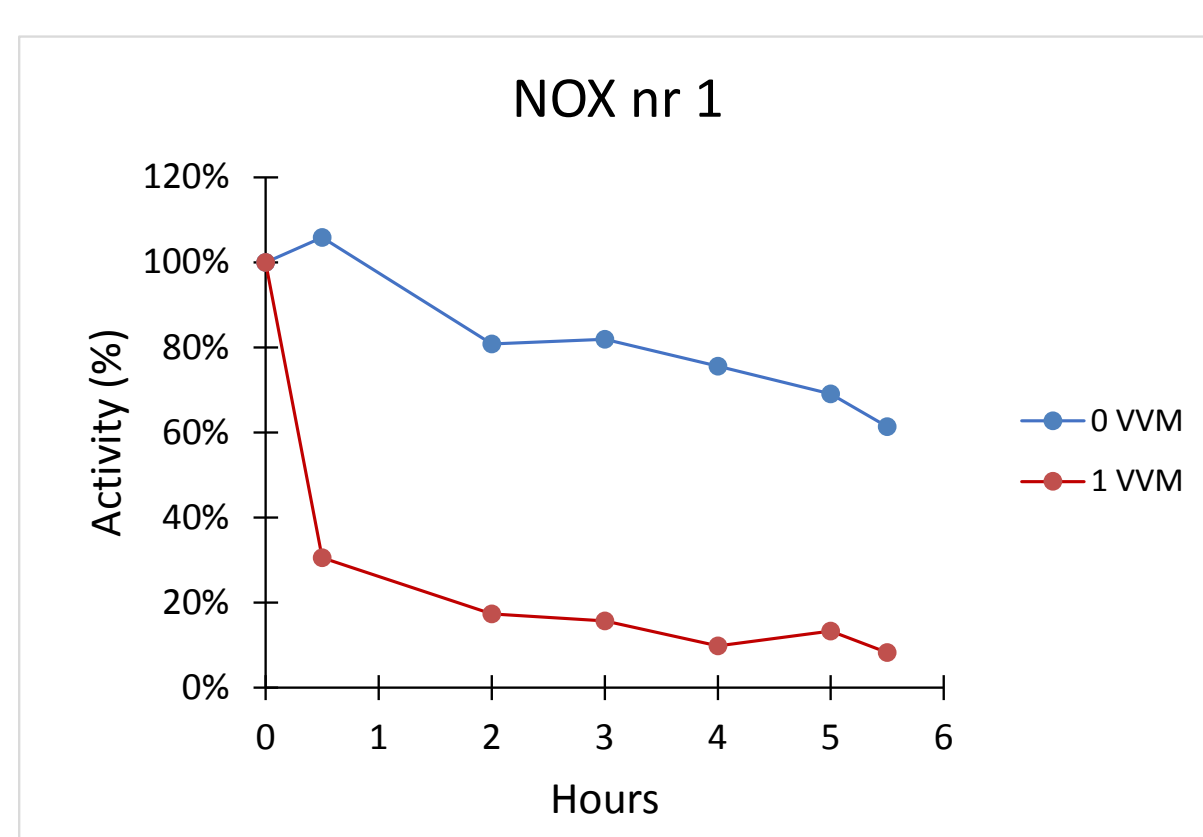
Oxygen supply is potentially problematic due to the sensitivity of the NOX enzyme. Here we investigate the effect of bubbling using N<sub>2</sub> to avoid effects related to oxidation of the NOX enzyme.

## Preliminary characterization:

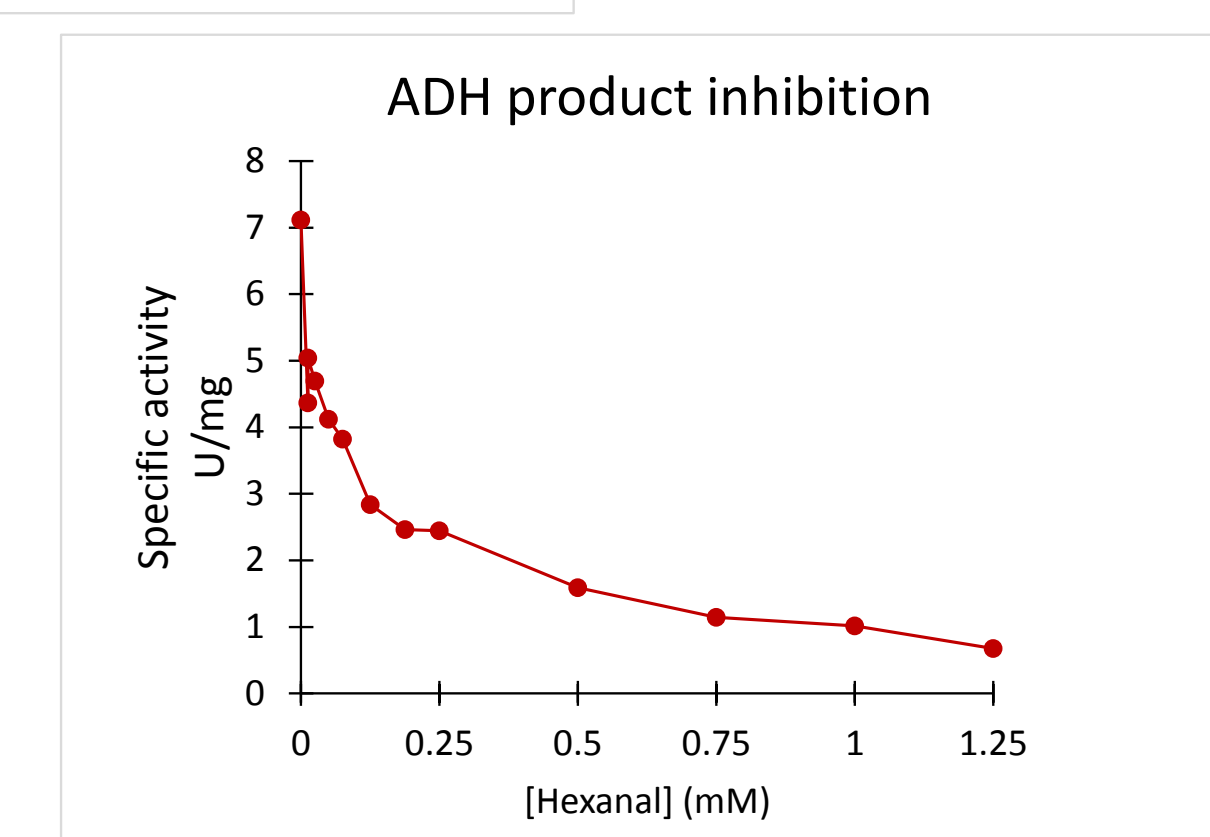
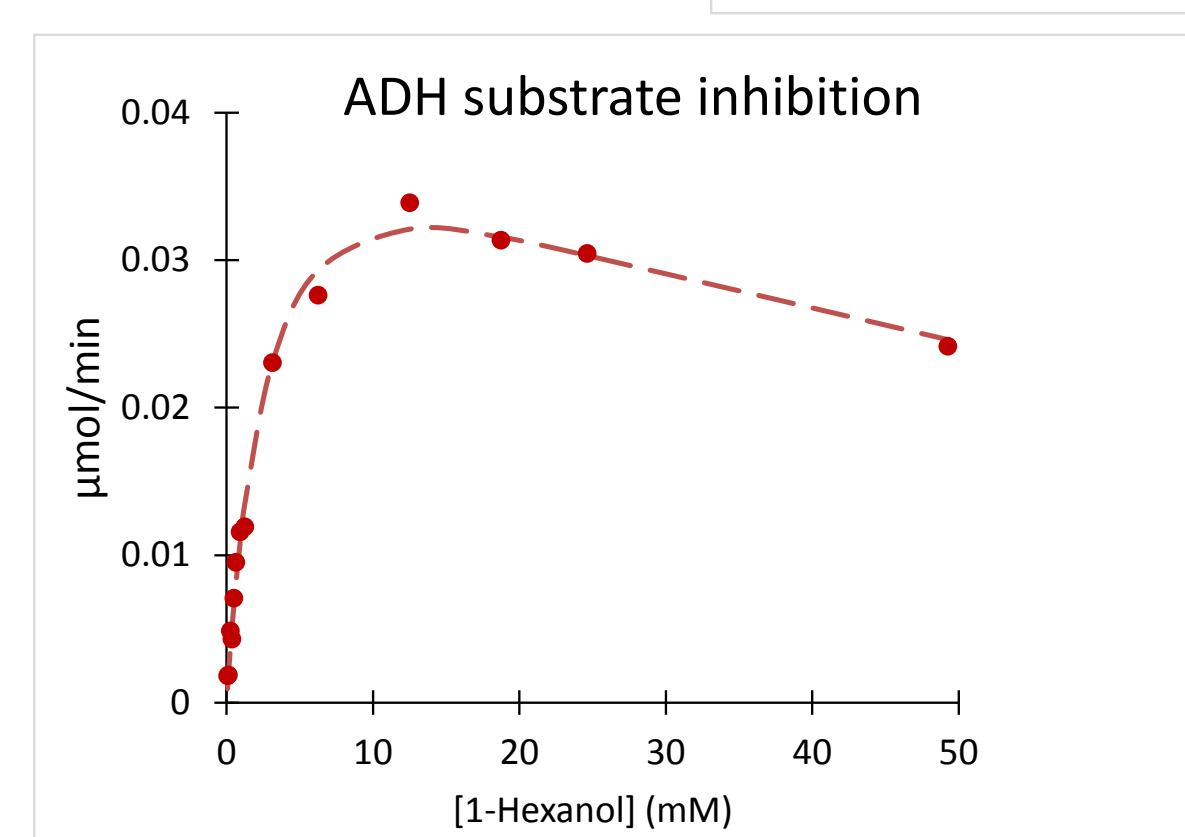
Several water forming NOXs are currently investigated in our lab. Preliminary data highlights several challenges for process implementation regarding both the NOX and the ADH.



Retaining a high NOX activity at alkaline pH is important for efficient enzyme use. An alkaline pH is beneficial for the ADH activity.



NOX nr 2 and 4 seem comparatively more resistant to the gas-liquid interface. Selecting a more robust NOX enzyme is important in order to supply oxygen effectively. Bubbling is the most straightforward oxygen supply method.



Both enzymes (ADH/NOX) can be severely inhibited by the alcohol substrate and the aldehyde product. This shows that characterizing the inhibition of both enzymes is highly important, and suggests that systems for substrate feeding and *in-situ* product removal may be necessary.

## Conclusions

- Characterization of the enzymes under process relevant conditions is needed.
  - For example, the alcohol substrate and aldehyde product inhibit both enzymes.
- A two-phase system could be beneficial as a substrate reservoir / product sink, and will be explored further.
  - Combining a two-phase system with effective oxygen supply presents a challenge.
- NOX-enzymes displayed different tolerance to the gas-liquid interface.
  - This property is important to investigate, since it affects the choice of oxygen supply method.

## References

- [1] B.Z. Zhan, A. Thompson, Tetrahedron 60 (2004) 2917.
- [4] M. Sudar, Z. Findrik, M.V. Domanovac, Đ. Vasić-Rački, Biochem. Eng. J. 88 (2014) 12.



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"Developing a validated technology platform for the application of oxygen dependent enzymes in synthesis and transformation of alcohols"